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# Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of *N*-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes

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**An acetyltransferase-overexpressing strain of *Salmonella typhimurium* (NM2009) has been used to investigate roles of human liver microsomal cytochrome P450 (P450) enzymes in the activation of carcinogenic nitrosamine derivatives, including *N*-nitrosodialkylamines and tobacco-smoke-related nitrosamines, to genotoxic products. Studies employing correlation of activities with several P450-dependent monooxygenase reactions in different human liver samples, inhibition of microsomal activities by antibodies raised against human P450 enzymes and by specific P450 inhibitors, and reconstitution of activities with purified P450 enzymes suggest that the tobacco-smoke-related nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and *N*-nitrosonornicotine (NNN) as well as *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) are oxidized to genotoxic products by different P450 enzymes, particularly P450 2E1 and 2A6. The activation of NDMA and NNN by liver microsomes was suggested to be catalyzed more actively by P450 2E1 than by other P450 enzymes because the activities were well correlated with NDMA *N*-demethylation and aniline *p*-hydroxylation in different human samples, and purified P450 2E1 had the highest activities in reconstituted monooxygenase systems. The relatively high contribution of P450 2A6 to the activation of NDEA and NNK was supported by the correlation seen with coumarin 7-hydroxylation in human liver microsomes, and antibodies raised against P450 2A6 inhibited both activities by ~50%. P450 3A4, 2D6 and 2C enzymes appear not to be extensively involved in the activation of these nitrosamines as judged by several criteria examined. Thus, this work indicates that several P450 enzymes, particularly P450 2E1 and 2A6, catalyze metabolic activation of nitrosamine derivatives including *N*-nitrosodialkylamines and tobacco-smoke-related nitrosamines in human liver microsomes.**

## Introduction

There is considerable evidence to support the view that carcinogenic *N*-nitrosamine derivatives are important factors in human cancer, through ingestion by smoking as well as food (1–3). Chemical mechanisms of carcinogenesis by these nitrosamines in humans are therefore of considerable interest (1–5). Numerous studies have shown that most of the nitrosamine derivatives are oxidized initially by cytochrome P450 (P450*) enzymes to yield reactive alkyldiazohydroxides (followed by possible formation of diazonium ions) that alkylate nucleic acids irreversibly (1,2,6). Very recently we have obtained evidence that acetylation of alkyldiazohydroxides by acetyltransferase may also be involved in the activation process because of the high sensitivity with nitrosamines and the model compound *N*-nitrosomethylacetoxymethylamine observed in acetyltransferase-overexpressing strain *Salmonella typhimurium* NM2009 (7). Oxidation by P450 enzymes is the key step in the activation of nitrosamines, and efforts have been made to identify P450 enzymes involved in these reactions (7–10).

P450 2E1 has been suggested to be the major enzyme catalyzing the *N*-dealkylation of short-chain *N*-nitrosamines in several animal species, including man (6,11–14). However, until recently it remained unclear whether P450 2E1 or other P450 enzymes are actually involved in the metabolic activation of nitrosamines in biological systems such as the Ames/*Salmonella* mutagenicity assay (14,15). Since the dose of nitrosamines required to induce reverse mutations in the Ames assay has been reported to be very high, it is difficult to define the roles of P450 enzymes in the reactions in this assay system (16–18). We have recently obtained evidence in rat liver microsomes that P450 2E1 is one of the major enzymes involved in the metabolic activation of *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) to genotoxic products in the tester strain *S.typhimurium* NM2009, which has high acetyltransferase activity (7). However, it is not known which P450 enzymes in humans catalyze activation of nitrosamine derivatives.

In this study we examined the roles of P450 enzymes in the activation of NDMA and NDEA and tobacco-smoke-related nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and *N*-nitrosonornicotine (NNN) in human liver microsomes using the newly developed tester strain *S.typhimurium* NM2009 (7,19). The results of correlations between P450-linked monooxygenase activities in 12 different human liver microsomal samples, inhibition of activities by specific antibodies and by chemical P450 inhibitors, and reconstitution of activities with purified human P450 enzymes suggest that different P450 enzymes, particularly P450 2E1 and 2A6, can catalyze the metabolic activation of these *N*-nitrosamines to genotoxic products. Roles of other P450 enzymes including P450 1A2 and 3A4 are also reported to be considerable.

## Materials and methods

*Materials*

NDMA and NDEA were purchased from Wako Pure Chemicals Co., Osaka, and Tokyo Chemical Industry Co., Tokyo respectively. NNK, NNAL and NNN were kindly donated by Dr L.A.Peterson, American Health Foundation (Valhalla, NY). Diethyldithiocarbamate and 4-methylpyrazole were obtained from Katayama Chemical Industry Co., Osaka, quinidine HCl from Sigma Chemical Co., St Louis, MO, and 7,8-benzoflavone from Aldrich Chemical Co., Milwaukee, WI. NADP+, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Tokyo. All other chemicals and reagents used were of the highest quality commercially available.

*Abbreviations: P450, liver microsomal cytochrome P450; NDMA, *N*-nitrosodimethylamine; NDEA, *N*-nitrosodiethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN, *N*-nitrosonornicotine.

*Enzyme preparations and antibodies*

Human liver samples were obtained from patients (denoted as HL-15, HL-45, HL-47, HL-48, HL-49, HL-51, HL-52, HL-53, HL-54 and HL-56) undergoing liver resection (20,21) or from organ donors (denoted as HL-114 and HL-125) through Tennessee Donor Services (Nashville, TN) (12,22). Human liver microsomes were prepared by the methods described (7,23).

Methods for purification of human P450 enzymes and the nomenclature used have been described previously (22–26). The specific contents (nmol of P450/mg of protein) of P450 1A2, P450 2A6, $P450_{MP}$ [one of the P450 2C enzymes that catalyze polymorphic *S*-mephenytoin 4-hydroxylation (26)], P450 2E1 and P450 3A4 thus purified were 5.3, 16.0, 13.7, 7.0 and 12.8 respectively. Good substrates for these P450 enzymes have been reported to be ethoxyresorufin and phenacetin for P450 1A2, coumarin for P450 2A6, *S*-mephenytoin for $P450_{MP}$, aniline, NDMA and chlorzoxazone for P450 2E1, and nifedipine and ethylmorphine for P450 3A4 (22–26). Antisera against these P450 enzymes were all obtained as described previously (22–25).

Rabbit liver NADPH-P450 reductase and cytochrome $b_5$ were purified to electrophoretic homogeneity using the basic method described by Yasukochi and Masters (27) and Taniguchi *et al.* (28) respectively.

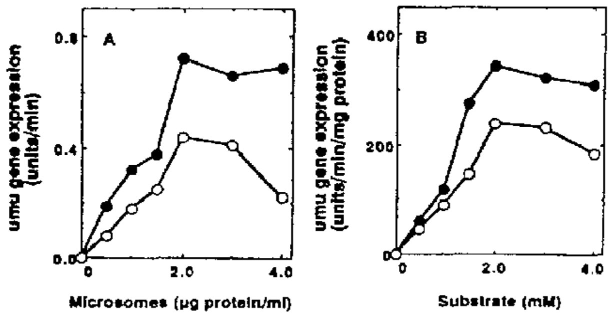


**Fig. 1.** Effects of concentrations of proteins (A) and substrate (B) on the activation of NDMA (●) and NDEA (○) catalyzed by liver microsomes (human sample HL-114). In (A) the substrate concentration used was 2 mM with varying concentrations of liver microsomes, and in (B) 2 µg/ml of microsomal protein was included in the reaction mixture. The expressed *umu* gene expression was calculated by subtracting the blank values, which were obtained by inactivating liver microsomes with heating at 100°C for 2 min. There were no increases in inducing *umu* gene expression in blank values when higher substrate concentrations were used.

*Metabolic activation of nitrosamine compounds by P450*

In this study we used a newly developed tester strain *S.typhimurium* NM2009 rather than *S.typhimurium* TA1535/pSK1002 (7) to detect genotoxic activities of reactive nitrosamine metabolites by measuring induction of *umu* gene expression in the bacterial cells. The former strain was constructed by introducing an *O*-acetyltransferase gene into the latter strain; the newly developed strain can express *O*-acetyltransferase activity at high levels (19). The tester strain used was kindly donated by Dr Y.Oda of Osaka Prefectural Institute of Public Health. Further studies demonstrating the usefulness of this system in the detection of various procarcinogens and promutagens (including NDMA and NDEA) in this newly developed strain have been reported recently (7,19).

The metabolic activation of nitrosamines to genotoxic metabolites by P450 was determined as described (7,20). Briefly, liver microsomes were generally added to give a final concentration of ~2 µg of protein/ml in the presence of the bacteria. Reconstituted P450 systems used to replace microsomes included 2 nM purified P450, 5 nM rabbit cytochrome $b_5$, 50 nM NADPH-P450 reductase, a mixture of lipids (10 µg/ml) consisting of dioleoylphosphatidylcholine, L-α-phosphatidyl-L-serine, L-α-1,2-dilauroyl-*sn*-glycero-3-phosphocholine (1:1:1) and sodium cholate (100 µg/ml). Incubations with 2 mM concentrations of nitrosamines were carried out for 120 min and expressed β-galactosidase activity was determined by the method of Miller (29). The induction of *umu* gene expression by activated carcinogens is generally presented as units of β-galactosidase activity/min/mg of protein (or nmol of P450).

*Other assays*

*N*-Demethylation of NDMA and ethylmorphine and *p*-hydroxylation of anilir were determined by the methods of Nash (30) and Guengerich (31) respectively. Ethoxyresorufin *O*-deethylation, chlorzoxazone 6-hydroxylation and coumarin 7-hydroxylation were assayed according to the methods as described (12,22,32). P450 and protein contents were assayed by the methods of Omura and Sato (33) and Lowry *et al.* (34) respectively.

## Results

*Activation of NDMA and NDEA by human liver microsomes*

Human liver microsomes were incubated with NDMA or NDEA in the presence of an NADPH-generating system and a bacterial tester strain *S.typhimurium* NM2009 and the induced *umu* gene expression due to DNA damage by the reactive metabolites of these procarcinogens was determined (Figure 1). The induction of *umu* gene expression, as determined by β-galactosidase activity, increased linearly with microsomal protein concentrations up to 2 µg/ml or with substrate concentration up to 2 mM with NDMA or NDEA. When the higher concentrations of

**Table I.** P450-dependent activation of nitrosamines and hydroxylation of drugs in different human liver microsomal samples

| Substrate | Human liver sample | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HL-15 | HL-45 | HL-47 | HL-48 | HL-49 | HL-51 | HL-52 | HL-53 | HL-54 | HL-56 | HL-114 | HL-125 |
| Metabolic activation of nitrosamines | | | | | | | | | | | | |
| NDMA[a] | 253 | 227 | 80 | 40 | 47 | 40 | 86 | 6 | 73 | 13 | 334 | 280 |
| NDEA[a] | 193 | 80 | 40 | 113 | 180 | 200 | 146 | 200 | 120 | 227 | 240 | 246 |
| NNK[a] | 282 | 54 | 82 | 82 | 206 | 237 | 136 | 175 | 196 | 244 | 303 | 296 |
| NNAL[a] | 190 | 221 | 131 | 124 | 265 | 124 | 110 | 162 | 37 | 169 | 158 | 149 |
| NNN[a] | 138 | 151 | 110 | 45 | 95 | 134 | 89 | 117 | 82 | 131 | 227 | 196 |
| Hydroxylation of drugs | | | | | | | | | | | | |
| Ethoxyresorufin[b] | 211 | 8.7 | 8.3 | 3.8 | 16 | 8.3 | 14 | 110 | 3.0 | 20 | 127 | 145 |
| Coumarin[c] | 24 | 0.59 | 0.45 | 0.32 | 6.9 | 0.46 | 15 | 21 | 1.4 | 107 | 112 | 117 |
| Aniline[d] | 0.74 | 0.74 | 0.77 | 0.84 | 0.84 | 0.21 | 0.35 | 0.28 | 0.14 | 0.31 | 8.1 | 6.3 |
| NDMA[e] | 0.56 | 0.28 | 0.44 | 0.25 | 0.98 | 0.29 | 0.38 | 0.38 | 0.28 | 0.46 | 5.1 | 4.0 |
| Ethylmorphine[e] | 0.28 | 0.35 | 0.41 | 0.24 | 1.20 | 0.22 | 0.22 | 1.73 | 0.47 | 0.12 | 0.97 | 0.71 |
| P450[f] | 0.60 | 0.19 | 0.34 | 0.20 | 0.87 | 0.23 | 0.25 | 0.45 | 0.11 | 0.24 | 0.53 | 0.51 |

Each value represents the mean of duplicate determinations. The standard deviation (range) in each value was < 15% of the mean.
[a]*umu* gene expression (units/min/mg protein).
[b]*O*-Deethylation (pmol resorufin formed/min/mg protein).
[c]7-Hydroxylation (pmol 7-hydroxycoumarin formed/min/mg protein).
[d]*p*-Hydroxylation (nmol *p*-aminophenol formed/min/mg protein).
[e]*N*-Demethylation (nmol HCHO formed/min/mg protein).
[f]Total P450 content (nmol/mg protein).

microsomal protein or substrate were included in the reaction mixture, the enhancement of *umu* gene expression was decreased, probably reflecting the cytotoxic responses by reactive metabolites of NDMA and NDEA to the bacteria. From these results the following experiments were done at these optimized conditions.

*Determination of P450-linked monooxygenase activities and comparison with activities that catalyze metabolic activation of several nitrosamines in liver microsomes of different human samples*

Ethoxyresorufin *O*-deethylation, coumarin 7-hydroxylation, aniline *p*-hydroxylation, NDMA *N*-demethylation and ethylmorphine *N*-demethylation have been reported to be catalyzed mainly by P450 1A2, 2A6, 2E1, 2E1 and 3A4 respectively in human liver microsomes (6,9–12,14,20,21,24). In order to see which P450 enzymes are the major ones involved in the metabolic activation of NDMA, NDEA, NNK, NNAL and NNN, we determined these activities and compared them with the activities in liver microsomes from different human samples (Table I). Total P450 contents of these microsomes are also presented. The activities varied widely among the different human samples examined. The most extensive variation in P450-linked monooxygenase activity was observed for coumarin 7-hydroxylation, followed by ethoxyresorufin *O*-deethylation and aniline *p*-hydroxylation. The variations in activities that catalyze activation of nitrosamines by liver microsomes were less dramatic: generally ~5-fold differences were seen in these cases except for activation of NDMA.

To examine the roles of individual human P450 enzymes in the activation of nitrosamines, we further determined the correlation coefficients for these monooxygenase activities in 12 different human samples (Table II). NDMA activation by liver microsomes was correlated well with NNN activation, aniline *p*-hydroxylation and NDMA *N*-demethylation; the latter two reactions have been shown to be catalyzed by P450 2E1 in humans (11). On the other hand NDEA activation correlated best with NNK activation and coumarin 7-hydroxylation; the latter has been reported to be catalyzed almost exclusively by P450 2A6 in human liver microsomes (22). The NNAL activation tended to correlate only with the total P450 content in human liver microsomes. Ethylmorphine *N*-demethylation activity did not correlate well with other monooxygenase activities except for the total P450 content in liver microsomes.

*Effects of anti-P450 antibodies and P450 inhibitors on the activation of nitrosamines by human liver microsomes*

The above results suggested that P450 2E1 and 2A6 enzymes may have major roles in the activation of nitrosamine derivatives in human liver microsomes. We further examined the effects of antibodies raised against human P450 enzymes on the metabolic activation of nitrosamines by human liver microsomes (Figure 2). Anti-P450 2E1 inhibited all of the nitrosamine activations by 50–60% in human liver microsomes. The inhibition by anti-P450 2E1 was most notable in the activation of NDMA and NNN; good correlations were seen in these two activities in different human samples as described above (Table II). Anti-P450 2A6 was also effective in inhibiting the activation of nitrosamines by liver microsomes, but the response was weaker than seen with anti-P450 2E1 except for the reactions with NDEA (Figure 2B). Anti-P450 1A2 showed weak but significant inhibition of the reactions with NDMA, NDEA, NNK and NNAL. Anti-P450 3A4 weakly inhibited the activation of NDMA, NNK and NNAL. None of the reactions examined were inhibited by anti-$P450_{MP}$.

Diethyldithiocarbamate and 4-methylpyrazole have been reported to be potent inhibitors for P450 2E1 (7,12,35), and in this study we confirmed that both chemicals inhibited strongly the P450 2E1-catalyzed chlorzoxazone 6-hydroxylation in human liver microsomes (Figure 3A). However, the effects of these chemicals on the P450 2A6-catalyzed coumarin 7-hydroxylation were found to be different; diethyldithiocarbamate inhibited the activity markedly, while 4-methylpyrazole enhanced the coumarin 7-hydroxylation in human liver microsomes (Figure 3B). The mechanism by which 4-methylpyrazole enhances the coumarin 7-hydroxylation is not clear at present. 7,8-Benzoflavone also inhibited the coumarin 7-hydroxylation, but quinidine did not affect the microsomal coumarin 7-hydroxylation; the former chemical is a specific inhibitor for P450 1A enzymes and the latter is for P450 2D6-catalyzed reactions (21,36). To demonstrate the roles of human P450 enzymes in the activation of nitrosamines the effects of these inhibitors were examined (Figure 4). Diethyldithiocarbamate strongly inhibited all of the nitrosamine activations examined, and 4-methylpyrazole was also inhibitory, but weaker than the diethyldithiocarbamate, for most of the reactions catalyzed by human liver microsomes. 7,8-Benzoflavone inhibited effectively the activations of NDMA, NDEA and NNK but not those of NNAL and NNN. However, none of the reactions examined were inhibited by quinidine.

**Table II.** Correlation coefficients (*r*) for P450-dependent activities with nitrosamines and drugs in different human liver microsomal samples[a]

| | NDEA[b] | NNK[b] | NNAL[b] | NNN[b] | Ethoxyresorufin[c] | Coumarin[d] | Aniline[e] | NDMA[f] | Ethylmorphine[f] | P450[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| NDMA[b] | 0.25 | 0.40 | 0.19 | 0.78 | 0.65 | 0.48 | 0.77 | 0.74 | −0.01 | 0.28 |
| NDEA[b] | | 0.89 | 0.18 | 0.56 | 0.58 | 0.73 | 0.51 | 0.57 | 0.31 | 0.43 |
| NNK[b] | | | 0.05 | 0.61 | 0.66 | 0.69 | 0.54 | 0.61 | 0.18 | 0.47 |
| NNAL[b] | | | | 0.26 | 0.22 | 0.09 | 0.07 | 0.09 | 0.32 | 0.69 |
| NNN[b] | | | | | 0.59 | 0.73 | 0.80 | 0.81 | 0.20 | 0.30 |
| Ethoxyresorufin[c] | | | | | | 0.48 | 0.51 | 0.52 | 0.31 | 0.53 |
| Coumarin[d] | | | | | | | 0.75 | 0.78 | 0.10 | 0.22 |
| Aniline[e] | | | | | | | | 0.99 | 0.26 | 0.35 |
| NDMA[f] | | | | | | | | | 0.31 | 0.41 |
| Ethylmorphine[f] | | | | | | | | | | 0.59 |

[a]The correlation coefficients were calculated from the results of Table I.
[b]*umu* gene expression (units/min/mg protein).
[c]*O*-Deethylation (pmol resorufin formed/min/mg protein).
[d]7-Hydroxylation (pmol 7-hydroxycoumarin formed/min/mg protein).
[e]*p*-Hydroxylation (nmol *p*-aminophenol formed/min/mg protein).
[f]*N*-Demethylation (nmol HCHO formed/min/mg protein).
[g]Total P450 (nmol P450/mg protein).

*Reconstitution of activation of nitrosamines in purified P450 monooxygenase systems*

The metabolic activation of nitrosamines to genotoxic products in *S.typhimurium* NM2009 was reconstituted using purified human P450 enzymes in the presence of NADPH-P450 reductase, cytochrome $b_5$, phospholipids and sodium cholate (Table III) (20,37). In support of the above data, P450 2E1 was found to be the most effective in all of the reactions catalyzing metabolic activation of nitrosamines. P450 2A6 could also catalyze activation of nitrosamines but was always somewhat weaker than P450 2E1. P450 1A2 had significant activities towards *N*-nitrosamines; but $P450_{MP}$ and P450 3A4 gave lower activities for activation of NDMA, NDEA and NNK.

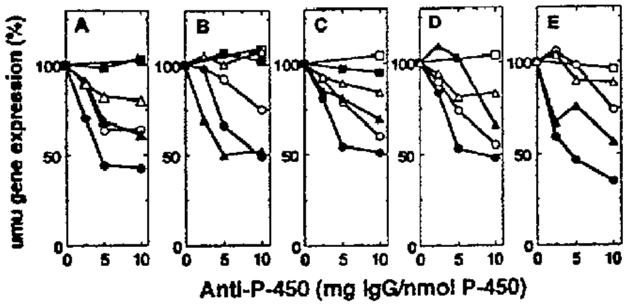


**Fig. 2.** Effects of anti-human P450 antibodies on the activation of NDMA (A), NDEA (B), NNK (C), NNAL (D) and NNN (E) catalyzed by liver microsomes (human sample HL-114). Antibodies raised against P450 1A2 (○), anti-P450 2A6 (▲), anti-$P450_{MP}$ (■), anti-P450 2E1 (●) and anti-P450 3A4 (△) were used (the effects of anti-$P450_{MP}$ were determined only in cases of NDMA, NDEA and NNK). The preimmune IgG (□) was also included as control. The specific activities without antibodies were 334 units/min/mg protien for NDMA, 234 units/min/mg protein for NDEA, 318 units/min/mg protein for NNK, 172 units/min/mg protein for NNAL and 263 units/min/mg protein for NNN.

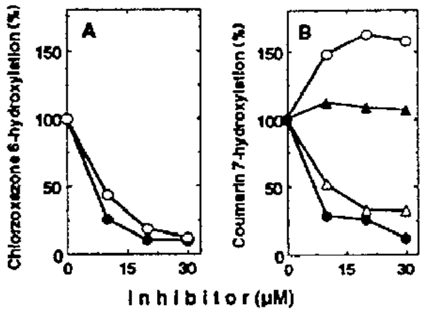


**Fig. 3.** (A) Effects of diethyldithiocarbamate (●) and 4-methylpyrazole (○) on chlorzoxazone 6-hydroxylation catalyzed by human liver microsomes (control activity: 3.4 nmol/min/mg protein). (B) Effects of diethyldithiocarbamate (●), 4-methylpyrazole (○), 7,8-benzoflavone (△) and quinidine (▲) on coumarin 7-hydroxylation catalyzed by liver microsomes (control activity: 0.16 nmol/min/mg protein). Liver microsomes from sample HL-114 were used.

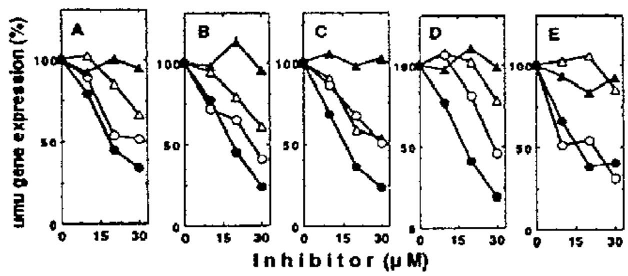


**Fig. 4.** Effects of 7,8-benzoflavone (△), quinidine (▲), 4-methylpyrazole (○) and diethyldithiocarbamate (●) on the activation of NDMA (A), NDEA (B), NNK (C), NNAL (D) and NNN (E) catalyzed by liver microsomes (human sample HL-114). The specific activities without inhibitors were the same as in the legend to Figure 2.

## Discussion

Nitrosamines have been shown to cause cancers at different sites of organs in experimental animals and should be considered as one of the most important groups of chemical carcinogens in the environment (1,2,38). For many years it has been recognized that humans are usually exposed to these procarcinogens through food or by the formation (of nitrosamines from nitrites and secondary amines) in the alimentary tracts (18,39). Numerous reports have appeared to suggest the importance of nitrosamines in human cancers through smoking; several nicotine-derived nitrosamines have been identified in cigarette smoke condensates and some of these nitrosamines are mutagens after metabolic activation (1,2). Initial oxidation of nitrosamines by P enzymes is believed to be critical in metabolic activation to cause cancer in man as well as in experimental animals (2,6). In rat experiments we reported previously that P450 2E1 is the major catalyst for the metabolic activation of NDMA and NDEA in *S.typhimurium* NM2009 (7).

Results presented in this study support the view that P450 2E1 is the most important enzyme in catalyzing metabolic activation of nitrosamines in human liver microsomes. We also find that P450 2A6 as well as P450 2E1 may be implicated in the activation of some of the nitrosamine derivatives as suggested by the following lines of evidence. First, the activities towards NDEA, NNK and NNN in different human liver microsomes correlate with the activities of coumarin 7-hydroxylation, a reaction known to be catalyzed by P450 2A6 in human liver microsomes (22,40). Second, polyclonal antibodies specific for human P450 2A6 (22) inhibited by ~50% nitrosamine activation catalyzed by human liver microsomes. Finally, purified P450 2A6 showed considerable activation of nitrosamines in a reconstituted monooxygenase system. P450 2A6 has recently been purified from human liver microsomes and identified as the mai-- coumarin 7-hydroxylase (22,40). In contrast to the orthologc rat P450 2A enzymes, the human counterpart P450 2A6 does not participate in the 7α-hydroxylation of testosterone (22,41). In this connection, mention should be made of our previous finding (7) that rat P450 2A enzyme may not be the major catalyst for the activation of NDMA and NDEA as opposed to the possible role of human P450 2A6 in the reactions (see above), and that such differences in nitrosamine metabolism may be one of the

**Table III.** Reconstitution of activation of several nitrosamine derivatives by purified human P450 enzymes[a]

| Substrate | *umu* gene expression (units/min/mol P450) | | | | |
|---|---|---|---|---|---|
| | P450 1A2 | P450 2A6 | $P450_{MP}$ | P450 2E1 | P450 3A4 |
| NDMA | 189 | 190 | 78 | 316 | 72 |
| NDEA | 133 | 194 | 56 | 211 | 111 |
| NNK | 111 | 205 | 78 | 339 | 17 |
| NNAL | 72 | 111 | – | 111 | – |
| NNN | 189 | 211 | – | 233 | – |

[a]Experiments for reconstitution of activation of nitrosamine derivatives were performed as described in Materials and methods. Each value represents the mean of duplicate determinations.

factors involved in species differences in chemical carcinogenesis.

The rates of activation of NNN showed good correlation with those of NDMA as well as activities of aniline *p*-hydroxylation and NDMA *N*-demethylation in different human liver microsomes (Table II). In contrast, NNK activation tended to resemble patterns of NDEA activation and of coumarin 7-hydroxylation. These results suggest that NDMA and NNN are activated more by P450 2E1 than by P450 2A6, and the opposite seems to be the case for NDEA and NNK. It should, however, be noted that the activities of coumarin 7-hydroxylation and NDMA *N*-demethylation showed good correlation ($r = 0.78$) in 12 human liver preparations; yet these two activities have been reported to be catalyzed by different P450 enzymes (6). In the same way the NNN activation showed good correlation with P450 2A6-supported-coumarin 7-hydroxylation as well as NDMA activation and NDMA *N*-demethylation. The reason why we obtained these contrasting results is not known at present, and we are currently investigating the experiments using more human samples. The activation of NNAL did not correlate with other parameters except for total P450 content in human liver microsomes, indicating that this procarcinogen may be catalyzed by several P450 enzymes in human liver microsomes.

P450 1A2 is also involved in part of the activation of some of the nitrosamines in human liver microsomes, because anti-P450 1A2 inhibited 20–40% of the activation of nitrosamines by human liver microsomes and the purified human P450 enzyme had considerable activities for the reactions. The contribution of P450 3A4 and 2C enzymes seems to be minor as judged by the results of correlation of activities, immunoinhibition and reconstitution of the nitrosamine activation. Although P450 2D6 has been suggested recently to play a role in the activation of NNK in a human B-lymphocyte cell line expressing human P450 enzymes (42), our results showing no significant effects of quinidine, a rather specific P450 2D6 inhibitor (36), on the nitrosamine activation by human liver microsomes suggest that the contribution of P450 2D6 for the reactions should be minor. More detailed experiments will be needed to address the epidemiological studies showing correlations between increased lung cancer risk and the ability to metabolize debrisoquine, one of the typical substrates for P450 2D6, in humans (43–45). However, the lack of correlations between these parameters has also been reported by other investigators (46,47). Mention should be made of our previous results, which indicate that P450 2D6 may not contribute to the activation of procarcinogens such as aromatic amines and nitrosamines in the extracts of cigarette smoke condensates in human liver microsomes (48).

The conclusion that human P450 2E1 seems to be favored over P450 2A6 [previously called P450 IIA3 by Crespi *et al.* (42,49)] in the activation of NDMA and vice versa for NDEA is consonant with the results seen by Crespi *et al.* (42,49) in comparing the mutagenicity at the *HGPRT* locus in human lymphoblast cells expressing the two cDNAs. It should be emphasized that the endpoint measured here is bacterial SOS response, and genotoxicity in one assay may not necessarily be reflected in another. The other system suggests activation of NNK in the order of P450 2A6 ~ 1A2 > 2D6 > 2E1 (42), which was not seen in the present study (Figures 2 and 4). NNK is transformed via several pathways, including the formation of methyldiazohydroxide (50), and there is evidence that it may be the one most related to lung cancer in A/J mice (51). In human liver microsomes P450 1A2 and P450 2E1 appear to contribute to NNK *N*-demethylation (50). The other product of the pathway leading to methyldiazohydroxide, 4-oxo-1-(3-pyridyl)-1-butanone, was recovered at a lower level and no inhibition was seen with any of the antibodies—this finding may be a reflection of the sensitivity of that assay. In a similar vein, measurable production of this keto-aldehyde was not detectable above the basal level with any of the expressd P450s in the cell system used. Nevertheless, it is not possible to state with certainty which adducts are responsible for the various genetic endpoints at this time.

In conclusion the present work clearly shows that several nitrosamine compounds could be catalyzed to genotoxic products by P450 enzymes, particularly P450 2E1 and 2A6, in human liver microsomes. The interindividual variations in the contents of these P450 enzymes may affect susceptibility to the procarcinogens in the environment.

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